Nucleoside conformations. XVII. A PMR study of 2'-anhydronucleosides and comparison with X-ray data

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ABSTRACT

A series of 2'-cyclo-nucleosides (2,2'-O-anhydro-uridine, -N₃-uridine and cytidine and 8,2'-S-anhydro-guanosine) have been studied by PMR in DMSO and D₂O. As expected these compounds are quite rigid, but their solution conformation is considerably different from that observed in single crystal x-ray studies. While the pyrimidine cyclonucleosides in the crystal form show a C₄'-endo conformation (pseudorotational phase angle P=212° to 233°), their solution conformation is C₁'-exo (P=130° to 138°) and the cyclothioguanosine shows a closely similar one (P=112°). Exocyclic rotamer distribution is different in the various compounds.

INTRODUCTION

In numerous papers on the solution conformation of nucleosides¹⁻⁵ the generally used description is that of a "rapid time average blend of conformers". Since the conformation is thus rarely that found in crystals, Altona and Sundaralingam^{6,7} have attempted to correlate PMR and crystal data by describing the solution conformation as a mixture of the N (3'-endo-2'-exo) and S (3'-exo-2'-endo) forms. Apparently, nucleosides the conformation of which is held fixed by an additional ring in another but the usual N and S conformers, like cyclonucleosides cannot be analysed in this way. On the other hand, such fixed nucleosides may be good reference compounds to correlate x-ray data with dihedral angles, obtained from proton-proton coupling constants. Unfortunately, the recent NMR and x-ray study of 2,5'-O-anhydro-2',3'-isopropylidene-uridine⁸ shows rather poor agreement in this respect. These differences may be due to solvent effects which could stabilize a solution conformation different from that in the crystal.

The recent publication of two x-ray studies of 2,2'-anhydrouridine^{9,10} and that of 2,2'-anhydrocytidine¹¹ prompted us to investigate several cyclonucleosides by PMR. After completion of this work a similar study by PMR of 2,2'-O-anhydro-uridine has appeared¹² the conclusions of which are in some details different from ours.

MATERIAL AND METHODS

2,2'-O-anhydro-uridine (compound IA, fig.1) was synthesised according to published procedures^{13,14}. Its 3'-acetate (IB) was a side product during the preparation of 2'-fluorinated nucleosides^{15,16}. It was obtained by acetylation¹⁷ of monomethoxytrityl-2,2'-O-anhydro-uridine. The 5'-position was deprotected with cold 1% trifluoroacetic acid in dichloroethane at 0° for 30min.

Fig.1: Structural formulae of cyclonucleosides studied.

3',5'-di-O-acetyl-2,2'-anhydro-uridine (IC) was prepared directly from IA by standard procedures¹⁷. 2,2'-O-anhydro-N³-uridine (II) was prepared¹⁸ by Dr. Kotick (Elkhart, Indiana) and generously donated. 2,2'-O-anhydro-cytidine (III) was prepared as in the literature¹⁹, as well as 8,2'-S-anhydro-guanosine²⁰.

PMR spectra were recorded at 25° except when otherwise stated on a CAMECA TSN-250 MHz spectrometer. All chemical shift measurements are relative to an internal TMS standard in DMSO and an internal DSS standard in D₂O.

RESULTS

A characteristic PMR spectrum that of compound IC in DMSO is shown in fig. 2. All peaks were sharp and in no case significant line broadening was observed. The similarity of the chemical shifts of the various pyrimidine cyclonucleosides (Table I) evidences the closeness of the magnetic environment of the protons. The cyclisation between O² and O₂, and thus formation of a *syn* like structure gives rise to a large downfield shift of H₁, ($\Delta\delta=0.2$ to 0.7 ppm) and H₃, ($\Delta\delta=0.2$ to 0.8 ppm) compared with that of Urd ($\delta_{H_1}=5.90$, $\delta_{H_3}=4.22$) which resemble that of typical *syn* nucleosides, like m⁶Urd and m⁶Cyd²¹ or β -cyanuric acid²². The solvent effect in going from DMSO to water is most marked for the proton H⁵ (0.35 ppm downfield) and about 0.2 to 0.3 ppm downfield for the sugar protons. Note the absence of a solvent effect on proton H⁶. The acetate groups on the sugar shift the geminal proton 0.6 to 0.9 ppm downfield and the vicinal protons 0.3 to 0.5 ppm

Figure 2: 250 MHz PMR spectrum of compound IC (2,2'-anhydrouridine-3'5'-O-di-acetate in DMSO.

downfield as had been observed in the 2'-fluoro-2'dUrd-acetates¹⁶. Acetate resonances are only 0.2 ppm apart, indicating similar environment, taking into account the difference between primary and secondary carbons. Surprisingly, there is no difference in the chemical shifts of H_1 of II and that of I (and its derivatives), although the keto group is located above H_1 in the case of II.

The cycloguanosine IV (fig. 3, Table I) shows a strong upfield shift ($\Delta\delta=0.55$) of H_3 , indicating deshielding of this proton because of the *anti*-like conformation of this nucleoside.

The proton-proton coupling constants (Table II) confirm the similarity of the conformation of the pyrimidine cyclonucleosides (as well as the acetates), especially as far as the sugar conformation is concerned. There is only a small solvent effect on the dihedral angles of the sugar. The conformation of IV appears to be different from the rest of the compounds studied.

In contrast, the coupling constants involving the exocyclic sugar protons indicate large variations in the different compounds as well as considerable solvent effects. The rotamer distribution which is in favor of the *gauche-gauche* rotamer in D_2O for I and II (Table II) is shifted to the two other rotamers in DMSO. Temperature studies in DMSO show some surprising results (Figure 4, Table III). Only in IA equal distribution of the rotamers

Table 1: Chemical shifts of cyclonucleosides studied

Compound	solvent	H ₁	H ₂	H ₃	H ₄	H ₅	H _{5''}	H _{5'}	H ₆	ac ₃	ac ₅	OH ₃	OH ₅
IA	DMSO	6.35	5.24	4.42	4.10	3.31	3.20	5.89	7.90			5.93	5.03
	D ₂ O	6.59	5.51	4.70	4.43	3.61	3.59	6.24	7.98				
IB	DMSO	6.41	5.49	5.37	4.35	3.43	3.31	5.89	7.89	2.12			5.17
	D ₂ O	6.61	5.70	5.51	4.59	3.70	3.63	6.24	7.98	2.19			
IC	DMSO	6.44	5.56	5.35	4.61	4.12	4.01	5.94	7.94	2.13	1.90		
	D ₂ O	6.63	5.77	5.57	4.84	4.34	4.16	6.27	8.00	2.19	1.94		
II	DMSO	6.43	5.21	5.01	4.09	3.30		6.06	7.79			5.95	4.41
	D ₂ O	6.71	5.44	4.69	4.41	3.61	3.61	6.24	7.90				
III	DMSO	6.56	5.40	4.50	4.26	3.44	3.32	6.53	8.28				a
	D ₂ O	6.65	5.57	4.72	4.45	3.64	3.61	6.58	8.12				
IV	DMSO	6.29	4.78	4.34	3.93	3.47	3.38						a

a) lyophilised in D₂O before preparing solution in DMSO, protons exchanged

Figure 3: 250 MHz PMR spectrum of IV (2',8-S-anhydroguanosine) in DMSO

is approached at high temperature. In IB and IC the rotamer distribution does not change much in going from 25° to 120°C. This is particularly surprising with IB which has a free OH group in the 5'-position. It is also noteworthy that in all three cases the *gauche-trans* and *trans-gauche* rotamers have about equal probabilities.

On the other hand, no solvent effect was observed in III upon exocyclic

Table 11: Experimental spin-spin coupling constants J_{ij} , dihedral angles ϕ_{ij} ^a and exocyclic rotamer distributions P ^b of 2'-thiaydnucleosides studied.

Compound solvent		$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$	$J_{5'6'}$	$\phi_{1'2'}$	$\phi_{2'3'}$	$\phi_{3'4'}$	P_1	P_{11}	P_{111}	J_{56}	$J_{3'03'}$	$J_{5'05'}$	
IA	DMSO	5.7	0.2	1.9	4.6	5.8	-11.8	37°	95°	-112°	0.21	0.47	0.32	7.4	4.4	5.1
	D ₂ O	5.0	0.3	1.8	3.5	4.0	-12.8	42°	97°	-111°	0.57	0.25	0.18	7.2		
IB	DMSO	6.0	0.2	1.7	4.2	3.8	-12.0	35°	95°	-110°	0.51	0.22	0.27	7.4		4.9
	D ₂ O	5.0	0.2	1.9	3.1	3.2	-12.7	42°	95°	-112°	0.72	0.15	0.13	7.2		
IC	DMSO	5.7	0.3	1.9	4.8	3.9	-12.2	37°	97°	-112°	0.42	0.23	0.34	7.5		
	D ₂ O	5.1	0.6	2.0	4.1	3.2	-12.0	41°	101°	-113°	0.59	0.15	0.26	7.4		
II	DMSO	5.8	0.5	1.8	not resolved			36°	100°	-111°			6.8			
	D ₂ O	6.0	0.6	2.1	3.7	3.7	-12.0	35°	101°	-113°	0.58	0.21	0.21	6.7		
III	DMSO	6.0	0.5	1.5	2.8	3.2	-12.0	35°	100°	-109°	0.75	0.15	0.12	7.4		
	D ₂ O	5.8	0.6	1.6	3.0	3.0	-12.9	36°	101°	-110°	0.75	0.12	0.12	7.4		
IV	DMSO	6.3	2.3	4.0	5.6	5.8	-11.6	33°	115°	-124°	0.09	0.47	0.44	---		

a) computed from the modified Karplus equation $J_{ij} = 10.5 \cdot \cos^2 \phi_{ij} - 1.2 \cdot \cos \phi_{ij}$

b) computed from the formulae $J_{4'5'} = P_{1..J} + P_{11..J} + P_{111..J} +$
 $J_{4'5''} = P_{1..J} + P_{11..J} + P_{111..J}$

$$P_1 + P_{11} + P_{111} = 1$$

Table III: Effect of temperature upon exo-cyclic rotamer distribution of 2,2'-O-anhydro-uridine and its acetates in DMSO.

compound	temp. °C	J _{4'5'}	J _{4'5''}	P _I	P _{II}	P _{III}
IA	23	4.6	5.9	0.18	0.46	0.36
	50	4.7	6.0	0.15	0.49	0.36
	120	4.8	5.0	0.28	0.37	0.35
IB	25	4.3	3.7	0.53	0.19	0.28
	75	4.1	4.4	0.44	0.29	0.27
	125	4.5	4.3	0.41	0.28	0.32
IC	22	4.8	3.9	0.42	0.23	0.35
	70	4.7	3.8	0.44	0.22	0.34
	120	4.6	3.6	0.47	0.20	0.33

Figure 4: PMR spectrum of 5'-protons of compound IC in DMSO at different temperatures. The top spectrum shows the displacement of the HDO peaks by adding a small amount of D₂O.

rotamer distribution where the *gauche-gauche* contribution is largely favored (Table II), in contrast with the *anti* nucleoside IV which has virtually no *gauche-gauche* conformer contribution.

DISCUSSION

The study of rigid nucleosides by PMR was undertaken in the hope to extract exact reference values for the Karplus equation to define sugar geometries. X-ray studies should give the desired dihedral angles which should fit to a modified Karplus equation. This implies that x-ray diffraction studies yield indeed only one conformer. Although this appears to be the case for 2',3'-O-isopropylidene-2,0^{5'}-anhydro-uridine⁸ where the isopropylidene group would be expected to offer additional rigidity, the correlation between NMR and x-ray data is poor.

In the case of the 2,2'-O-anhydro-pyrimidine nucleosides the x-ray studies show the existence of two crystallographically independent molecules, which have similar, but not identical conformation⁹⁻¹¹. The bond angles between the two molecules can and do differ considerably (Table IV) (there is for instance a difference in the angle C₁-C₂-C₃-C₄ of 9°). This implies that these cyclonucleosides are less rigid than expected since they can assume more than one conformation in the crystal.

As shown in Table IV, the agreement between the dihedral angles computed from the spin-spin coupling constants using the modified Karplus equation^{6,7} with those obtained by crystallography is poor. On the other hand the observed coupling constants describe well the sugar conformation in solution and the agreement between lines (b) and (c) in Table IV is quite good. This indicates that the anhydronucleosides studied are behaving as rigid molecules in solution (flexible molecules, like the natural nucleosides or nucleotides show large differences between the two lines and r.m.s. values of 15° and more). The values P=131° to 138° computed for the solution conformation correspond to a C₁'-exo conformation, while the crystal structures with P=212° to 227° (II has P=233° in the crystal¹¹) are 4'-endo-3'-exo. Interestingly, the guanosine derivative IV shows a quite similar sugar conformation in solution as the cyclo-pyrimidine-nucleosides (P=112° corresponds to 1'-exo-0₁'-endo). This is easily accounted for by the slightly longer C-S bonds.

The absence of significant temperature effects on the sugar ring protons also observed by Cross and Schleich¹² favor the conclusion that the nucleosides are rigid.

The differences between the crystal and solution conformation observed

Table IV: Experimental (c) and computed (b) spin-spin coupling constants and dihedral angles of cyclo-nucleosides studied.

compound	solvent	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$\phi_{1'2'}$	$\phi_{2'3'}$	$\phi_{3'4'}$	r.m.s. ^a	P^b	τ_m^b	reference	comments
IA	x-ray	9.3	0.5	0.0	3.5	100.0	-91.2		227	29	(9)	molecule A
		8.8	0.0	0.0	12.7	90.4	-85.4		212	34	(9)	molecule B
	x-ray	9.3	0.5	0.0	3.5	100.0	-91.2		227	29	(10)	molecule A
		8.9	0.0	0.0	12.3	99.9	-85.2		213	35	(10)	molecule B
IA	DMSO (c)	5.7	0.2	1.9	37.2	95.5	-111.8			135	38	
	(b)	5.6	0.1	2.2	38.0	92.7	-113.8	1.9		135	38	
	D ₂ O (c)	5.0	0.3	1.8	41.4	97.0	-111.2			133	41	
	(b)	5.1	0.0	2.3	40.8	91.4	-114.6	3.8		133	41	
IB	DMSO (c)	5.0	0.2	1.7	35.4	95.5	-110.4			138	37	
	(b)	5.9	0.1	2.0	36.4	92.8	-112.4	1.9		138	37	
	D ₂ O (c)	5.0	0.2	1.9	41.4	95.5	-111.8			133	42	
	(b)	5.0	0.0	2.3	41.4	91.1	-114.7	2.9		133	42	
IC	DMSO (c)	5.7	0.3	1.9	37.2	97.0	-111.8			135	38	
	(b)	5.7	0.1	2.3	37.4	93.4	-114.3	2.5		135	38	
	D ₂ O (c)	5.1	0.6	2.0	40.8	100.7	-112.5			131	40	
	(b)	5.4	0.1	2.7	39.0	93.8	-116.7	4.8		131	40	
II	DMSO (c)	5.8	0.5	1.8	36.6	99.7	-111.2			135	36	
	(b)	5.9	0.2	2.3	35.9	94.4	-114.5	3.6		135	36	
	D ₂ O (c)	6.0	0.6	2.1	35.4	100.7	-113.2			132	35	
	(b)	6.1	0.3	2.6	34.7	96.1	-116.1	3.2		132	35	
III	DMSO (c)	6.0	0.5	1.5	35.4	99.7	-109.0			138	35	
	(b)	6.1	0.1	2.0	34.6	94.0	-112.5	3.9		138	35	
	D ₂ O (c)	5.8	0.6	1.6	36.6	100.9	-109.7			136	36	
	(b)	6.0	0.2	2.2	35.3	94.3	-113.7	4.5		136	36	
IV	DMSO (c)	6.3	2.3	4.0	33.5	114.5	-124.2			112	35	
	(b)	6.9	1.2	4.8	29.8	106.8	-128.3	5.5		112	35	

(a) r.m.s.: root-mean-square of the difference between observed (c) and computed dihedral angles ϕ_{ij} .

(b) computed with the aid of programme CONFOR^{6,7}, where P is the pseudorotational phase angle, τ_m the pucker of the sugar molecule. Tetrahedral geometry was assumed for the carbon atoms. The x-ray data of IA^{9,10} were treated in similar way and the dihedral angles and coupling constants computed using again tetrahedral carbon symmetry.

(c) obtained from the vicinal coupling constants using the modified Karplus equation as in Table II.

for this series of cyclonucleosides would little affect the H-¹³C couplings between C² and H₁, and C² and H₂, studied by Lemieux *et al.*²⁴.

It should be noted that the two crystallographic studies of IA^{9,10}

concluded on different sugar conformations. While Suck and Saenger⁹ name the conformation correctly C_4 , -endo- C_3 , -exo, Delbaere and James¹⁰ propose a C_2 , -endo- C_3 , -exo conformation, although the detailed data of both studies are virtually the same (see Table IV). In fact the use of programme CONFOR clearly shows that the 4'-endo-3'-exo is the only possible one.

The rotamer distribution around the exocyclic C_5 , is influenced by temperature and solvent changes. While in D_2O the *gauche-gauche* rotamer is favored this is not the case in DMSO, except for II. Selective binding of DMSO and/or steric interference between the 5'-acetate group (in the case of IC) and the perpendicular base may be responsible for this effect. The upfield shift of H^5 in DMSO may be an indication of a hydrophobic interaction between DMSO and the base. Such upfield shifts are also observed in dinucleotides¹⁸ and aromatic-aliphatic complexes¹⁹⁻²¹. Also, it should be noted that the x-ray diffraction studies of IA^{9,10} show a unusual *gauche-trans* orientation of the C_5 , - O_5 , bond.

It is not clear, however, why the conformer distribution shows little variation with temperature in IB and IC and is different from that of IA. Also in IC the rotamer distribution is not equilibrated at 120° C and far from the 1:1:1 distribution of the nonacetylated compound IA. Relatively large energetic barriers appear to be involved.

In contrast to Cross and Schleich¹² we have not observed significant line broadening in the compounds studied. These authors have evoked the possibility of long range coupling between the various ring protons. The coupling constants (J_{ij} = 0.5 to 0.8 Hz) are quite small and difficult to detect and can only be assigned by the use of the LAOCOON programme. Such a procedure may or may not be valid, since with a sufficient number of coupling constants any spectrum can be simulated by LAOCOON. Although the existence of long-range couplings cannot be excluded, this does not seem to be warranted by our experimental data. Furthermore, one would also expect such long range couplings with the base protons. The line width of the base protons does not support such couplings.

These studies clearly show that the cyclonucleosides can not be described by the canonical N (2'-exo-3'-endo) and S (2'-endo-3'-exo) forms. It is not surprising to find unusual coupling constants, which will not fit a N-S pseudo-rotamer distribution^{6,7}. Also the argument between crystallography and solution studies remains unsolved, since even rigid molecules may have more than one conformation, one which may be stabilized by crystal forces and

another one by solvation.

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